American Journal of Medical Genetics 66:209–215 (1996)

An Autosomal Dominant Triphalangeal Thumb: Polysyndactyly Syndrome With Variable Expression in a Large Indian Family Maps to 7q36

Uppala Radhakrishna, Jean-Louis Blouin, Jitendra V. Solanki, Ghanshyam M. Dhoriani, and Stylianos E. Antonarakis

Laboratory of Human Molecular Genetics, Department of Genetics and Microbiology, Geneva Medical School and Division of Medical Genetics, Cantonal Hospital, Geneva, Switzerland (U.R., J.-L.B., S.E.A.); Department of Animal Genetics and Breeding, Veterinary College, Anand, India (J.V.S., G.M.D.)

Hereditary developmental abnormalities of the upper or lower limbs in humans are easily recognizable phenotypes that can be used in the mapping and cloning of genes involved in normal human development. We studied a large Indian pedigree (UR002) with an autosomal dominant triphalangeal thumb (TPT) and polysyndactyly (PSD). The abnormalities were present only in the upper limbs, and the phenotype was fully penetrant. The expression of the phenotype was variable and ranged from unilateral TPT to bilateral TPT, preaxial du-, tri-, or quadruplication of the thumb, or syndactyly of multiple thumbs. There were 112 affected individuals in the pedigree. Previous linkage analyses on apparently similar phenotypes have identified a locus at 7q36 [Heutink et al., 1994, Nature Genet 6:287–291; Tsukurov et al., 1994]. To map the gene responsible for the TPT-PSD in family UR002, we performed linkage analysis in DNA from 47 affected and 7 normal individuals. Marker D7S550, located at 7q36, yielded a maximum LOD score of 11.31 at $\theta = 0.00$. Additional markers in the region also showed no recombination. These data indicate that the gene responsible for the hand abnormality in pedigree UR002 maps to the same region as that in previous pedigrees with similar phenotype. Further analyses of recombinants among all the linked families by using new polymorphic markers will narrow the critical genomic region and facilitate positional cloning of the elusive gene.

KEY WORDS: **preaxial polydactyly, triphalangeal thumb, autosomal dominant, chromosome 7q36, linkage analysis**

INTRODUCTION

Various malformations of digits are heterogeneous; they occur as isolated anomalies or as part of particular syndromes. The prevalance of polydactyly in the general population ranges from 0.37 to 1.2 in 1,000 [Ohkura, 1956; Neel, 1958; Woolf and Woolf, 1970; Castilla et al., 1973]. Preaxial polydactyly is manifested by additional fingers on the radial side of the hand and on the hallucal ray of the foot. In several cases, the expression of anomaly varies widely and has been classified as (1) polydactyly of thumb and or great toes (type 1), (2) polydactyly of a triphalangeal thumb (TPT) and/or duplication of great toes (type 2), (3) polydactyly of an index finger (type 3), and (4) polysyndactyly of thumbs and/or great toes (type 4) [Temtamy and McKusick, 1978]. Syndactyly is characterized by fusion of soft tissue and/or bones of fingers or toes. A reclassification of syndactylylies and polydactylies was suggested by Winter and Tickle [1993].

Recently, a TPT polysyndactyly (TPT-PSD) syndrome was mapped by linkage analysis in 4 pedigrees to the telomeric region of chromosome 7 (7q36) between polymorphic markers D7S550 and D7S594 [Heutink et al., 1994; Tsukurov et al., 1994; Hing et al., 1995]. The present study was undertaken to map the locus of an autosomal dominant phenotype that includes (with variable expression) triphalangeal thumbs and/or polysyndactyly (TPT-PSD) of hands in a large Indian family. Linkage analysis indicated that this syndrome also maps in the D7S550–D7S559 region of 7q36 and it is probably allelic with the previously described TPT locus [Heutink et al., 1994; Tsukurov et al., 1994; Hing et al., 1995].

Description of Family UR002

We have recently reported a large Indian family with preaxial polydactyly [Radhakrishna et al., 1993] from

Received for publication December 15, 1995; revision received February 22, 1996.

Address reprint requests to Stylianos E. Antonarakis, Centre Médical Universitaire, 1 rue Michel Servet, CH-1211 Genève, Switzerland. E-mail: sea@medsun.unige.ch

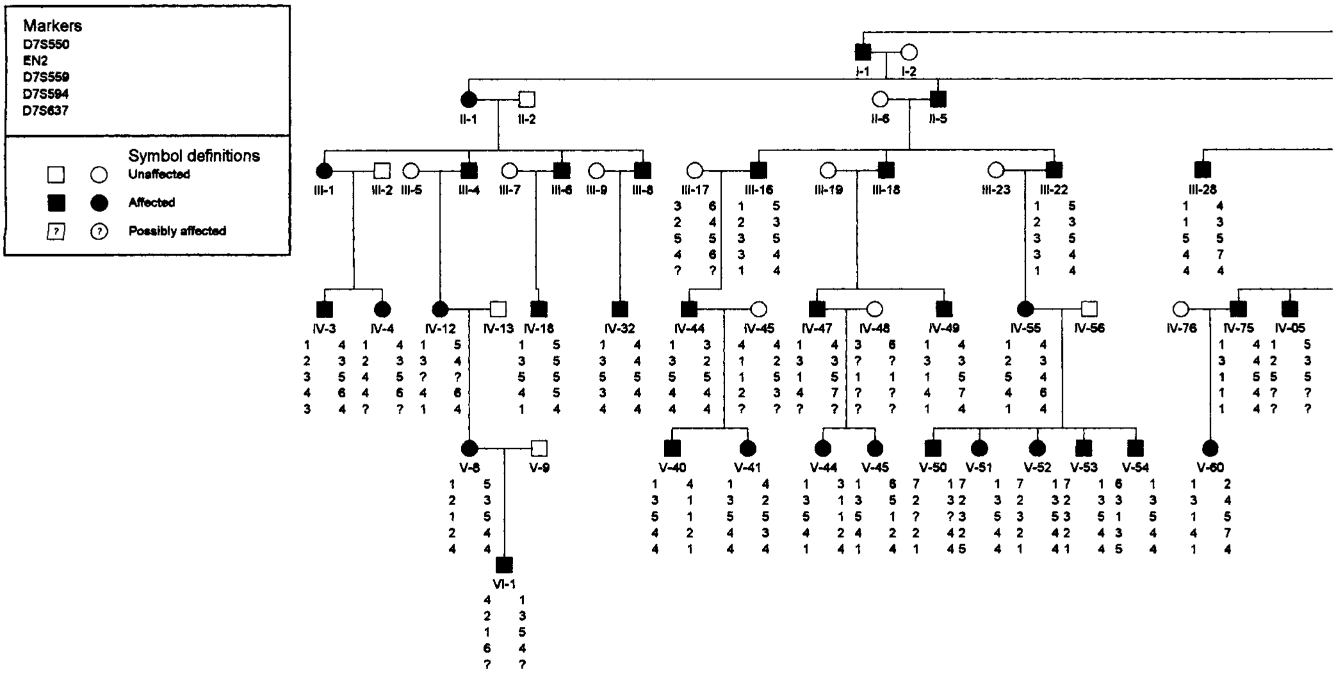

Fig. 1. Partial pedigree of family UR002 with triphalangeal thumb polysyndactyly (TPT-PSD). Genotypes for several linked polymorphic markers on chromosome 7q36 (D7S637, D7S550, EN2, D7S559, and D7S594) are shown.

Gujarat state. Subsequently, we have ascertained several additional families in the same region with pre- and postaxial polydactyly. The present family (UR002) with TPT-PSD, shown in Figure 1, is different from the Indian family reported by Radhakrishna et al. [1993]. The pedigree consists of 310 individuals including 112 affected (58 males and 54 females). The anomaly was present only in the upper limbs. More than 90 individuals are severely affected in one or both hands, with a high degree of phenotypic variation ranging from unilateral to bilateral TPT, preaxial duplication, triplication, or quadruplication of thumb, or syndactyly of multiple thumbs, hypoplastic additional finger, articulated TPT, additional finger, or duplication of TPT (Table I, Figs. 2–3). None of the individuals in the family had any foot anomalies. The less severely affected subjects showed unilateral hand malformation with small or rudimentary fingers. There was no consanguinity. The hand anomaly was disadvantageous in all severely affected individuals because of dependence on agriculture and social problems in females. Surgical correction was performed in a few individuals. The expression of the trait in the present family clearly supports autosomal dominant inheritance with variable expression. No skipping of generations was observed, with penetrance close to 100%.

More than 85 affected living individuals were diagnosed clinically. Peripheral blood samples were collected from 54 consenting relatives including 47 affected and 7 normal individuals. Clinical photographs were taken of each individual included in the linkage study. Hand radiographs were taken of selected individuals.

DNA Polymorphism and Linkage Analysis

Genomic DNA was purified from blood lymphocytes according to standard SDS–proteinase K and phenol/chloroform extraction methods. DNA polymorphisms were analyzed by polymerase chain reaction (PCR) amplification of simple sequence repeats. Microsatellite markers located in the 7q36 area (D7S637, D7S550, D7S559, D7S594, and EN2) were used first because of the previously identified linkage in pedigrees with similar phenotypes; 2 additional loci, D7S524, located at 7q11.2, and D6S296, were also studied [Genome Database (http://gdbwww.gdb.org); NIH/CEPH Collaborative Mapping Group 1992; Weissenbach et al., 1992]. One oligonucleotide primer of each marker was labeled with γ^{32}P-ATP with T4 polynucleotide kinase. PCR was performed on an MJ Research thermocycler to amplify from 150 ng of genomic individual DNA in a total volume of 15 μl mixture per reaction containing 0.4 pM of labeled forward primer, 2.6 pM of unlabeled reverse primer, 1.3 μM of each DNTP, and 0.25 U Taq polymerase. PCR products

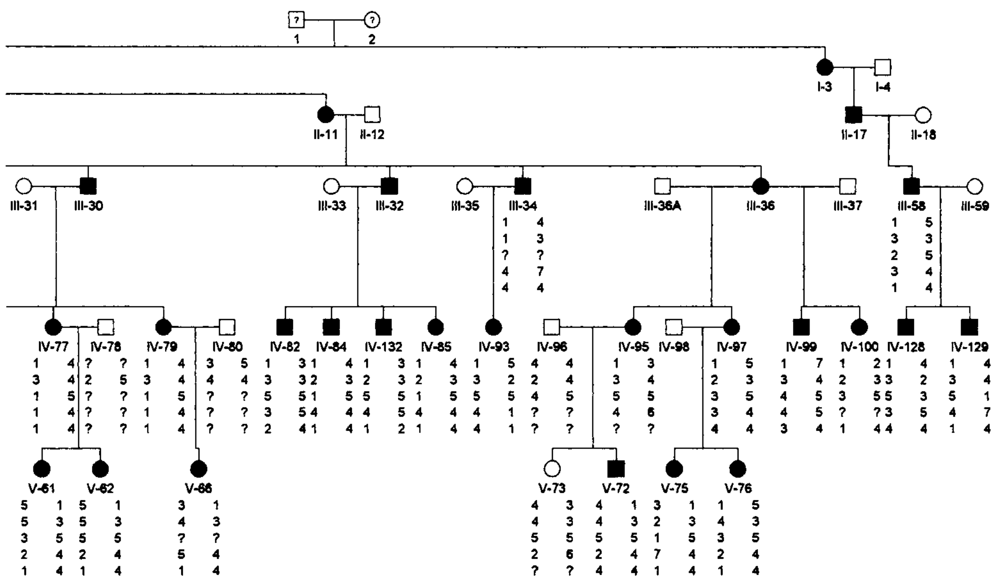

Fig. 1. (*Continued.*)

were separated by electrophoresis in a 6% denaturing urea/polyacrylamide gel.

Family information and marker genotypes were stored in the pedigree computer program Cyrillic®. Analysis was performed with the ILINK program in the LINKAGE software package [Lathrop et al., 1984]. Maximum LOD score was calculated for each marker locus by assuming autosomal dominant mode of inheritance with 100% penetrance. For all markers, the allele frequencies were kept equal.

RESULTS

Results of pairwise analyses between the phenotype of TPT-PSD family UR002 and 6 7q36 markers are shown in Table II. A maximum LOD score of 11.31 was obtained for marker D7S550 at recombination fraction $\theta = 0.00$ (Fig. 4). No recombination was observed with polymorphic markers D7S559 and EN2, which gave a significant LOD score of more than 7. However, recombination was observed between the phenotype and markers D7S637 and D7S594. The TPT-PSD locus in this family was therefore mapped in the EN2–D7S550–D7S559 region. Two additional markers that map outside to the 7q36 region, D6S296 and D7S524, were also tested; as expected, no evidence for linkage was observed.

DISCUSSION

The occurrence of preaxial polydactyly of fully developed triphalangeal digits in addition to the thumb, unilateral to bilateral TPT, preaxial duplication, triplication, or quadruplication of thumb, or syndactyly of multiple thumbs, hypoplastic additional finger, articulated TPT, additional finger, or duplication of TPT in members of the same pedigree suggests that the gene responsible for these abnormalities has variable phenotypic expression. Its autosomal dominant form of inheritance with high degree of penetrance is evident from the pedigree shown in Fig. 1.

Linkage analysis of the TPT-PSD phenotype in the large Indian family (UR002) presented here showed that the locus maps to 7q36 in the same interval (EN2–D7S550–D7S559) as that previously described [Heutink et al., 1994; Tsukurov et al., 1994; Hing et al., 1995] in pedigrees with similar phenotypes. Best linkage was obtained with marker D7S550 at no recombination with disease locus (LOD score = 11.31). The mutation responsible for the phenotype in family UR002 can therefore be allelic to that of the previous studies.

The manifestations of the families with TPT-PSD in the 3 previously published reports of linkage to chromosome 7q36 markers include triphalangeal thumbs with hypoplastic thenar muscle and occasional syndactyly [Heutink et al., 1994], pre- and postaxial polydactyly with severe syndactyly of hand and feet [Tsukurov et al., 1994], and extra biphalangeal thumb and duplication of great toe without syndactyly [Hing et al., 1995]. Thus, the phenotypes range from a small skin tag to TPT with polysyndactyly of the hand and duplication of toes. Different mutations in the locus on 7q36 may cause different variations of the phenotype; alternatively, the modification of the phenotype can be due to other gene products that are involved in the development of hands and feet. There is also the possibility that this locus includes a cluster of genes, each of which is involved in various stages of hand/foot development.

No recombination was observed with a polymorphic marker adjacent to gene EN2 at 7q36 [Logan et al., 1978; Poole et al., 1989], which is a human homolog of the engrailed-2 gene of *Drosophila* [Kornberg, 1981]. Engrailed-2 is involved in axial patterning in

TABLE I. Summary of Findings in Affected Relatives With Triphalangeal Thumbs/Preaxial Polydactyly/Polysyndactyly Included in Linkage Analysis[a]

ID no.	Right hand	NFR	Left hand	NFL
IV-79	Normal	5	Duplication of thumb	5
V-66	TPT + Additional finger	(−) 6	TPT	(−) 5
IV-100	TPT + Hypoplastic finger	(−) 6	TPT + Hypoplastic finger	(−) 6
IV-75	TPT + Articulated finger + Rudimentary finger	7	TPT + Articulated finger + Hypoplastic finger	(−) 7
IV-82	TPT	5	TPT	5
IV-93	TPT + Hypoplastic finger	(−) 6	TPT + Hypoplastic finger	(−) 6
IV-84	TPT	5	TPT	5
III-34	TPT	5	Rudimentary articulated thumb	6
V-60	TPT	5	TPT + Articulated triphalengeal finger	7
III-28	Syndactyly of triphalengeal thumbs	6	TPT + Hypoplastic finger	(+) 6
IV-132	Syndactyly of 3 triphalengeal thumbs	7	Syndactyly of two triphalengeal thumbs	6
III-58	Duplication of thumb + Hypoplastic T finger	(−) 6	Duplication of thumb	5
IV-129	TPT + Syndactyly of additional finger	(+) 6	TPT + Syndactyly of additional finger	(+) 6
IV-128	TPT + Hypoplastic finger	6	TPT + Two hypoplastic fingers	(+−) 7
IV-77	Duplication of TPT	6	Duplication of TPT + Hypoplastic finger	(−) 6
V-62	TPT	5	TPT	5
V-61	TPT + Hypoplastic finger	6	TPT + Hypoplastic finger	6
IV-44	Duplication of TPT + Hypoplastic finger	(−) 6	TPT + Hypoplastic finger	6
IV-41	TPT	5	TPT	5
III-16	TPT + Hypoplastic finger	6	Normal	5
V-40	Duplication of thumb	5	Triplication of thumb + Hypoplastic finger	(−) 6
III-22	Triplication of thumb	5	Normal	5
IV-49	TPT + Additional finger	(+) 6	Additional triphalengeal finger	(−) 6
V-44	TPT + Hypoplastic finger	(−) 6	TPT + Hypoplastic finger	(−) 6
V-45	TPT + Hypoplastic finger	(−) 6	Duplication of TPT + Hypoplastic finger	(−) 6
IV-99	TPT	5	Articulated two additional hypoplastic fingers	(−) 7
IV-85	Syndactyly of TPT	6	Syndactyly of TPT	6
IV-95	Normal thumb + Fully grown articulated finger	(−) 6	Normal thumb + Fully grown articulated finger	(−) 6
V-72	TPT + Hypoplastic finger	(−) 6	Attriculated TPT	6
IV-97	TPT + Articulated additional thumb	6	TPT + Attriculated additional thumb	6
V-75	TPT + Hypoplastic fingers	(−) 7	TPT + Hypoplastic fingers	(−) 7
V-76	TPT + Hypoplastic fingers	(−) 7	TPT + Hypoplastic fingers	(−) 7
IV-03	Additional TPT	(−) 6	Syndactyly of TPT + Hypoplastic additional finger	(−) 7
V-52	Hypoplastic fingers	(−) 7	Hypoplastic fingers	(−) 7
V-51	TPT + Additional hypoplastic fingers	(−) 7	TPT + Additional Hypoplastic fingers	(−) 7
V-50	TPT + Hypoplastic finger	(+) 6	TPT + Hypoplastic finger	(+) 6
V-55	Normal	5	TPT	5
V-53	TPT + Hypoplastic finger	(+) 6	TPT + Hypoplastic finger	(+) 6
V-54	Syndactyly of TPT	(+) 6	Syndactyly of TPT	(+) 6
IV-12	Duplication of TPT	5	Duplication of TPT	5
VI-01	TPT + Hypoplastic finger	(+) 6	TPT + Two hypoplastic fingers	(+−) 7
V-08	TPT + Articulated hypoplastic TPT	(+) 6	TPT + Hypoplastic finger	(+) 6
IV-18	TPT	5	Duplication of thumb + Articulated finger	6
IV-32	TPT + Hypoplastic finger	(+) 6	Normal	5
IV-05	TPT + Hypoplastic finger	(−) 6	TPT + Hypoplastic finger	(−) 6
IV-69	TPT + Articulated thumb	(−) 6	TPT + Articulated thumb	(−) 6
IV-47	Duplication of TPT with syndactyly	5	Duplication of TPT with syndactyly	5

[a]ID no. = individual in the pedigree shown in Figure 1; TPT = triphalangeal thumb, NFR = number of fingers in the right hand, NFL = number of fingers in the left hand, (−) = location of the additional finger (preaxial to the existing finger), (+) = location of the additional fingers (presence between the thumb and second index finger).

Drosophila. Inactivation of this locus renders the animal incapable of maintaining segmental and compartmental borders; mutations in this gene lead to interruption of normal morphogenesis. Han and Manley [1993] documented that *Drosophila* En protein can act as a specific repressor of activated transcription. Two different genes, En-1 and En-2 of mouse, contain sequence similarity to the *Drosophila* engrailed gene [Martin et al., 1990]. En-2 maps to mouse chromosome 5, in the vicinity of hemimelic extra toe (Hx) and hammertoe (Hm) mutations [Joyner and Martin, 1987] that cause skeletal defects of all 4 limbs. Hx is a dominant mutation, characterized by preaxial polydactyly and hemimelia [Knudsen and Kochhar, 1981], whereas the

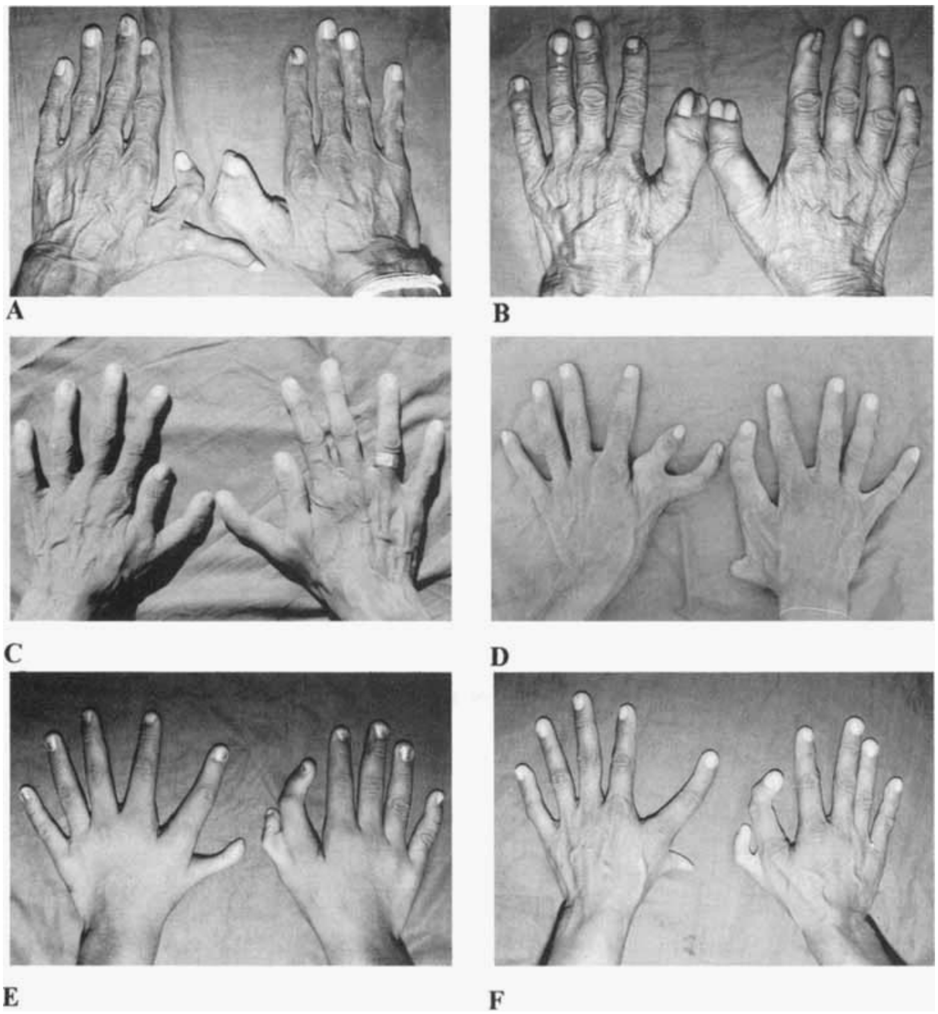

Fig. 2. **A–F:** Hand photographs of several affected individuals. Clinical description is given in Table I. Their position in the pedigree is shown in Figure 1.

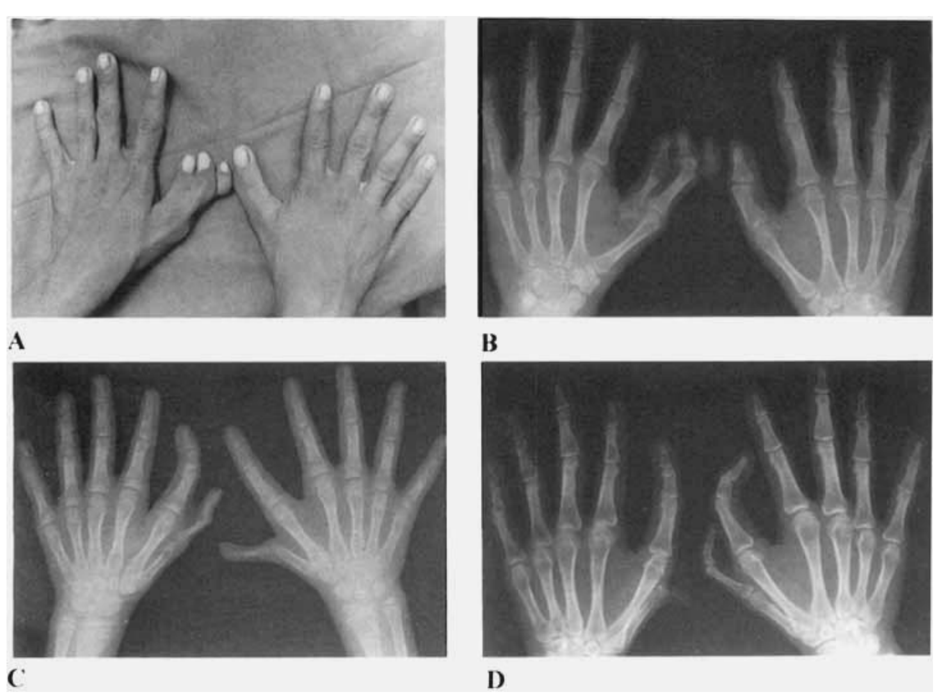

Fig. 3. **A:** Clinical photographs and (**B**) radiographs of the same affected individual with triplication of left thumb and normal right hand. **C–D:** X-ray radiographs of 2 different individuals with bilateral triphalangeal thumbs and hypoplastic triphalangeal thumbs.

TABLE II. Pairwise LOD Score Data Between the TPT-PSD Phenotype and Various Markers

Markers	Z_{max}	θ
Chromosome 7q36[a]		
D7S637	3.49	3.3
EN2	7.34	0
D7S550	11.31	0
D7S559	7.19	0
D7S594	8.51	4.0
Other markers		
D7S524	0.25	33
D6S296	0.29	69

[a]Microsatellite markers are arranged from centromere to telomere. The genetic distances among the different 7q36 markers are 4.3 cM from D7S637 to EN2, 2.3 cM from EN2 to D7S550, 4.1 cM from D7S550 to D7S559, and 4.1 cM from D7S559 to D7S594 [Tsui et al., 1995].

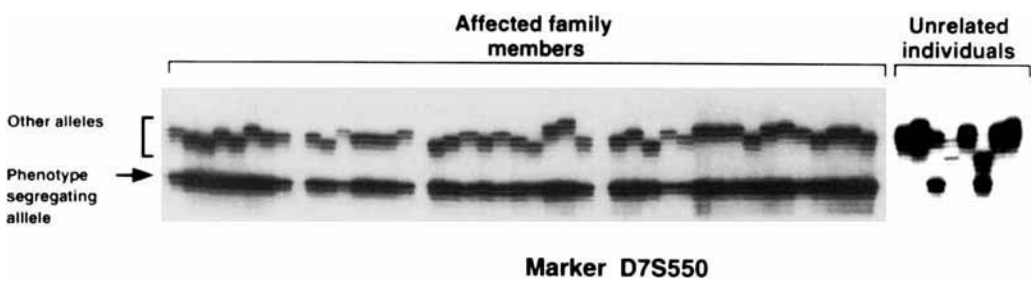

Fig. 4. Genotypes for marker D7S550 in family UR002 with TPT-PSD. All affected relatives share a common polymorphic allele for this marker.

semidominant mutation Hm, causes the failure of the webbing between the toes to undergo normal regression during development, resulting in the strong flexion of the second phalanx of digits on all 4 feet [Green, 1964]. These 2 mouse mutants are located very close to each other; however, they are nonallelic, with only 1 recombinant having been observed in 3,664 progeny of 2 different crosses [Sweet, 1982]. Because the engrailed gene is important for axial patterning in *Drosophila*, it remains a candidate for the phenotype in our family. However, this gene has been excluded as a candidate for the phenotype reported by Tsukurov et al. [1994].

Cloning and characterization of the gene responsible for our TPT-PSD by positional cloning and further analysis of the mutations in each family will provide a better understanding of normal human limb development.

ACKNOWLEDGMENTS

This study was supported by funds from the University and Cantonal Hospital of Geneva. We thank all members of this family for their cooperation and for donating blood samples for our study.

REFERENCES

Castilla E, Paz J, Mutchinick O, Munoz E, Giargiutti E, Gelman Z (1973): Polydactyly: A genetic study in South America. Am J Hum Genet 25:405–412.

Green MC (1964): Mouse News Lett 31:27.

Han K, Manley JL (1993): Functional domains of the *Drosophila* engrailed protein. EMBO J 12:2723–2733.

Heutink P, Zguricas J, Van Oosterhout L, Breedveld GJ, Testers L, Sandkuijl LA, Snijders P, Weissenbach J, Linkhout D, Hovius S, Oostra BA (1994): The gene for triphalangeal thumb maps to the subtelomeric region of chromosome 7q. Nature Genet 6:287–291.

Hing AV, Helms C, Slaugh R, Burgess A, Wang JC, Herman T, Dowton SB, Donis-Keller H (1995): Linkage of preaxial polydactyly type 2 to 7q36. Am J Med Genet 60:128–135.

Joyner AL, Martin GR (1987): En-1 and En-2, two mouse genes with sequence homology to the drosophila engrailed gene: Expression during development. Gene Dev 1:29–38.

Knudsen TB, Kochhar DM (1981): The role of morphogenetic cell death during abnormal limb-bud outgrowth in mice heterozygous for the dominant mutation hemimelia-extra toe (Hm). J Embryol Exp Morphol 65:289–307.

Kornberg T (1981): Engrailed: A gene controlling compartment and segment in *Drosophila*. Proc Natl Acad Sci USA 78:1095–1099.

Lathrop GM, Laloel JM, Julier C, Ott J (1984): Strategies for multilocus linkage analysis in humans. Proc Nat Acad Sci USA 81: 3443–3446.

Logan C, Willard HF, Rommens JM, Joyner AL (1978): Chromosomal localization of the human homeobox-containing Genes, En-1 and En-2. Genomics 4:206–209.

Martin GR, Richman M, Reinsch S, Nadeau JH, Joyner A (1990): Mapping of the two mouse engrailed-like genes: Close linkage of En-1 to dominant hemimelia (Dh) on chromosome 1 and of En-2 to hemimelic extra-toes (Hx) on chromosome 5. Genomics 6:302–308.

Neel JV (1958): A study of major congenital defects in Japanese infants. Am J Hum Genet 10:398–445.

NIH/CEPH collaborative mapping group (1992): A comprehensive genetic linkage map of the human genome. Science 258:67–86.

Ohkura K (1956): Clinical genetics in polydactylism. Jpn J Hum Genet 1:11–23.

Poole SJ, Law ML, Kao FT, Lau YF (1989): Isolation and chromosomal localization of the human En-2 gene. Genomics 4:225–231.

Radhakrishna U, Multani AS, Solanki JV, Shah VC, Chinoy NJ (1993): Polydactyly: A study of a five generation Indian family. J Med Genet 30:296–299.

Sweet HO (1982): Mouse News Lett 66:66.

Temtamy S, McKusick VA (1978): The genetics of hand malformation. Birth Def OAS 14:3–128.

Tsui L-C, Donis-Keller H, Grzeschik K-H (1995): Report of the second international workshop on human chromosome 7 mapping. Cytogenet Cell Genet 71:2–21.

Tsukurov O, Boehmer A, Flynn J, Nicolai JP, Hammel BCJ, Trail S, Zaleske D, Mankin HJ, Yeon H, Ho C, Tabin C, Seidman JG, Seidman C (1994): A complex bilateral polysyndactyly disease locus maps to chromosome 7q36. Nat Genet 6:282–286.

Weissenbach J, Gyapay G, Dib C, Vignal A, Morissette J, Milasseau P, Vaysseix G, Lathrop M (1992): Second-generation linkage map of the human genome. Nature 359:794–801.

Winter RM, Tickle C (1993): Syndactylies and polydactylies: Embryological overview and suggested classification. Eur J Hum Genet 1:96–104.

Woolf CM, Woolf RM (1970): A genetic study of polydactyly in Utah. Am J Hum Genet 22:75–78.